Chapter 8

FOSSIL TINTINNIDS

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8.1 INTRODUCTION: A PAUCITY OF DATA

Ciliates are difficult to observe and interpret, even when seen through the lenses of powerful microscopes. Although they have a relatively high number of morphological characters compared with other microbial eukaryotes, this number is nevertheless extremely limited and their simplified structures may easily be converged upon by distantly related lineages (Dunthorn & Katz 2008). The processes of fossilization and the slow decay of time commonly affect those few morphological characters that allow us to place fossils into extant taxa with any reliable resolution, or even to accurately identify them as ciliates. The very characters that we use to circumscribe extant species and larger clades – the patterns of somatic and oral cilia, the arrangements of kinetosome-associated microtubules and fibers, and morphogenesis – are obscured or lost in ciliate fossils. Soft cell parts may be preserved in ciliates, but only under exceptional conditions such as in amber (Ascaso et al. 2005; Martín-González et al. 2008; Schmidt et al. 2001; Schönborn et al. 1999; Waggoner 1994).

Tintinnids, with their more easily fossilized loricae, therefore offer the best hope of tracing ciliate evolution throughout the long history that is preserved in the rock record. However, these loricate fossils only contain two pieces of evidence to both determine they are tintinnid ciliates and to place them somewhere in the tintinnid taxonomy: their shape and their composition. With these two pieces of evidence, numerous paleontologists and taxonomists have analyzed putative tintinnid fossils since their discovery in the late 19th century by Rüst (1885). These “tintinnids” have been reported from Proterozoic through Recent marine deposits. The only other group of heterotrophic plankton that has nearly such an early beginning is the Radiolaria, which appeared in the early Cambrian period (Braun et al. 2007; Nazarov 1973) and diversified throughout it (Dong et al. 1997; Won & Below 1999) to the present. Proterozoic microplankton chiefly includes a variety of acritarchs, which are cysts of phytoplankton (Lipps 2006). Benthic heterotrophic microbial eukaryotes may have been present in the Proterozoic (Huldtgren et al. 2011) and certainly began to radiate in the Cambrian–Ordovician (Lipps 2006), but the other major groups of microplankton, including diatoms, foraminifera, coccolithophorids, and silicoflagellates, appeared in the Mesozoic era and are extant today. Against this general background, the presence of tintinnids in Proterozoic and Paleozoic rocks cannot be predicted.

Known, extant tintinnids now number about 1200 described species in 75 genera both in marine and brackish- to freshwater environments (Agatha 2009); only about 10 species live in freshwaters (Tappan 1993). They occur in today’s oceans in five separate biotas: cosmopolitan occurrences of hyaline loricate forms, neritic occurrences of organic and agglutinated loricae in nearshore waters, warm-water-containing the most numerous species of tintinnids, and boreal and austral forms in high northern and southern areas (Chapter 10). Tintinnids may be especially abundant in nearshore eutrophic waters (Xu et al. 2011) and were even found in caves on land that had been penetrated by marine waters (van Hengstum & Scott 2011).

Fossil tintinnids are almost exclusively marine and number far fewer described taxa. The fossils are difficult to observe and interpret, and mistakes have commonly been made. Many of these so-called tintinnids from the rock record have been described or reported from cross-sections observed in petrographic thin-sections of the rock. There these fossils may resemble tintinnid cup-like loricae, but so do cross-sections many other tiny fossils or parts of fossils, especially in limestones of the Paleozoic and Mesozoic, making positive identification trying, if not impossible. Others of these tintinnid fossils are demonstrably the remains of other kinds of eukaryotes that have undergone diagenetic changes. Still others have thickly calcified skeletons, unknown in modern forms that have been assigned to other groups, although many workers accept them as tintinnids. For these reasons, fossil tintinnids have been controversial and hard to relate to modern forms and to the evolutionary history of the group and to ciliates in general. Figure 8.1 shows all claimed tintinnid occurrences in the geologic record. However, all such reports require special scrutiny. Extensive historical summaries of these analyses are found elsewhere (Tappan & Loeblich 1968; Tappan 1993), and we summarize more recent work here.

8.2 PROTEROZOIC TINTINNIDS: NO EVIDENCE OF A BEGINNING

Molecular clock analyses place the origin of ciliates sometime in the Proterozoic (Berney & Pawlowski 2006; Douzery et al. 2004; Parfrey et al. 2011; Wright & Lynn 1997). If these estimates are correct, then
ciliate fossils dating from those ancient times are possible. Because tintinnids appear to relatively evolved ciliates, we might not expect them to be found among the earliest plankton. However, three sets of fossils have been described and interpreted as Proterozoic tintinnids so far.

The oldest supposed fossil tintinnids were reported from the very old (Figs. 8.1 and 8.2) Mesoproterozoic Huangmailing Formation, China, dated at 1600 million years ago (MYA) (Li & Zhan 2006; Li et al. 2009). Eight species described in six genera have the general shape of unaltered and undistorted modern tintinnid loricae (Fig. 8.2). If these are truly tintinnid fossils, then the date for the origins of ciliates would be pushed back much further in time than any molecular clock estimate, and would imply an even older origin for the ciliates in general. However, because they come from high-grade metamorphic rocks, termed “granulite” and “leptite” by Li & Zhan (2006) and Li et al. (2009), these specimens should have been affected to some degree by metamorphism. Given the absence of any of those kinds of metamorphic effects, these so-called fossils are more likely to be contaminants or mineral shards from the metamorphic rock itself. They could also have come from parts of the rock that were not affected by any metamorphic processes; however, Li & Zhan (2006) and Li et al. (2009) are not clear about whether these unaltered areas in the rocks exist.

The second set of fossils (Figs. 8.1 and 8.3) comes from limestone in the Tsagaan Oloom Formation in Mongolia, dating to 635–715 MYA (Bosak et al. 2011). Many of the over 100 organic fossils appear flask-shaped with an apical blunt-end collar, tapering to a bowl-shaped base. However, in some of the figures (Bosak et al. 2011), the fossils are not flask-shaped; rather, the basal end looks like a slightly larger version of the apical blunt end (Fig. 8.3b). On the surface of some of the fossils are alveolar spheroids (not to be confused with the flattened alveolar sacs found in ciliate cell membranes) that are 2–5 μm in diameter; these spheres are potentially similar to those found in some tintinnids during lorica formation (Gold & Morales 1976b).

An additional character shape described from these fossils is the central invaginations in the apical collars (Fig. 8.3c) that are “consistent with a cemented or collapsed aperture” (Bosak et al. 2011). Presumably, they are composed of the same organic material as the rest of the fossils. However, in extant tintinnids, no such
Central-invagination-shaped structures occur in or on the loricae, and the collars are broadly opened to allow the cells to feed. Although closing apparatuses occur in some tintinnids such as Codonella, these structures are found at the base of the collar, and their membranelles, which are 0.1–0.2 μm thick, are arranged like an iris diaphragm (Agatha 2010a). The membranelles may be secreted by the cell, but their composition is different from the loricae. Such thin membranes are unlikely to be preserved in any fossil. The overall organic compositions of the Mongolian fossils (Bosak et al. 2011) do support a tintinnid relationship. In addition, silicon was also found in these fossils, an element not found in extant ciliates without agglutinated loricae; whether this silicon was included during the fossilization process is not known. Given that extant tintinnids do not have central invaginations of the collar of their loricae and silicon is lacking in non-agglutinated species, these fossils are unlikely to be tintinnids. In addition, given the lack of such central invaginations in the tests of testate amoebae, reliable taxonomic placement within the eukaryotes is currently impossible.

The youngest set comes from the 580 MYA Wengan Phosphate Member of Doushantuo Formation of China (Figs. 8.1 and 8.4). These rocks, formerly thought to be marine but now considered a lake deposit (Bristow et al. 2009), preserve an array of microfossils said to include sponge and other smaller animal embryos and larval bilaterians (Chen et al. 2009); embryonic stages larger than 16 cells were attributed to taphonomic factors that eliminated them, perhaps because of their fragility (Dornbos et al. 2005). These so-called animal embryos are neither animals nor embryos, but more likely represent other microbial eukaryotes (Huldtgren et al. 2011). The bilaterian fossils have been dismissed as well because the putative cell layers are diagenetic overgrowths on interior and exterior surfaces (Bengtson & Budd 2004) likely of and on acritarch cysts (Dunthorn et al. 2010). From these same rocks, two new tintinnid taxa, Eotintinnopsis pinniforma and Wujiangella beidoushanese (Fig. 8.4), were described based on single specimens (Li et al. 2007). These fossils were interpreted as putatively cup-shaped organisms with organic loricae, characters that are consistent with extant tintinnids. However, their similarity to tintinnid shape ends there. In the specimen of Eotintinnopsis (Fig. 8.4a–c), multi-tiered apical and a subapical feather-like structures were described. These apical features are unknown in any extant ciliate, and, like the misidentified bilaterians from the same rocks, this fossil likely represents the remains of two acritarchs that were altered, distorted, and compacted together during and after fossilization (Dunthorn et al. 2010). Of the two putative Doushantuo fossil tintinnids, Wujiangella (Fig. 8.4d–f) is the most similar to extant ciliates (Li et al. 2007). However, its so-called somatic cilia are twice as wide as they should be, and they lack any kinety arrangement that would normally be found in extant tintinnids (Dunthorn et al. 2010). Rather than being cilia, the projections on Wujiangella are most likely spines on diagnostically and taphonomically
Fig. 8.3 Putative tintinnid fossils from the 635–715 MYA Tsagaan Oloom Formation of Mongolia from Bosak et al. (2011). (a, b) Side view with deflated round bowls, constricted necks, and centrally invaginated collar. a is ~130 μm wide; b is 70 μm long. (c) View of apical central invagination. c is 75 μm long. © Geological Society of America.

Fig. 8.4 Putative tintinnid fossils from the 580 MYA Wengan Phosphate Member of Doushantuo Formation of China from Li et al. (2007). (a) Eotintinnopsis pinniforma: longitudinal section; scale bar, 50 μm. (b) Close up of feather-like membranelles on E. pinniforma: scale bar, 10 μm. (c) Reconstruction of E. pinniforma. (d) Wujiangella beidoushanese: longitudinal section; scale bar, 50 μm. (e) Close up of cytostome and cytopharynx of W. beidoushanese: scale bar, 10 μm. (f) Reconstruction of W. beidoushanese. somatic cilia; cb, cell body; cp, cytopharynx; cs, cytostome; ft, feather-like membranelles; l, lorica; m, membranelles; p, peduncle. © The Geological Society of London.
degraded acritarchs (Dunthorn et al. 2010). A third ciliate, reportedly a suctorian, was also described from one specimen as *Yonyangella ovalis*. It too is a diagenetically and taphonomically altered acritarch. This evidence and the freshwater depositional environment of the Doushantou Formation indicate that these fossils are not tintinnids: rather they are acritarch cysts of algal microplankton.

Thus, given the alternative interpretations by Dunthorn et al. (2010) and herein of the fossils of Li et al. (2007), Bosak et al. (2011), Li & Zhan (2006), and Li et al. (2009), no solid evidence of Proterozoic tintinnids or other ciliates comes from the Precambrian rock record. Darwin’s dilemma of the lack of fossils for this ancient age (Schopf 2001; Knoll 2004) therefore still holds for at least the ciliates. If there are tintinnid fossils from this ancient time, they have yet to be discovered.

8.3 PALEOZOIC TINTINNID REPORTS: TOO THIN TO KNOW

In the Phanerozoic eon, tintinnid fossils have been reported rarely in Paleozoic rocks, but are much more commonly reported in the Mesozoic era where they can be quite abundant. Whether or not any of these fossils are tintinnids is much debated.

Tintinnids have been reported from thin sections in Ordovician carbonate rocks (Fig. 8.5) of the Sahara of Africa (Chennaux 1968), the Silurian of Spain (Hermes 1966), the Lower Devonian of North Africa (Cuvillier & Sacal 1963) and of Canada (Murray & Taylor 1965), and the Mississippian of Teverga in northern Spain (Cuvillier & Barreyre 1964). From only one of these occurrences, the Devonian, was a new genus and species described. Thus Tappan & Loeblich (1968), although noting the reports of tintinnids in Ordovician, Silurian, and Devonian, did not include them in their review because new taxa were not described. The Mississippian occurrence (Cuvillier & Barreyre 1964) are not tintinnids and are more likely calcispheres (Tappan & Loeblich 1968), which are spherical calcite objects found commonly in carbonate rocks and believed to be algal in origin. The other records include two-dimensional cross-sections of spheres and cups, some with an opening and flange. Although the cross-sections do resemble those of Mesozoic calpionellids, they have not been confirmed as tintinnids by the study of free specimens. So far, no Paleozoic tintinnids are known with certainty.

8.4 MESOZOIC TINTINNIDS AND CALPIONELLIDS: SAME SHAPE, DIFFERENT COMPOSITION

Tintinnids and the calpionellids have been described from the upper Triassic to lower Cretaceous sedimentary rocks from both onshore and offshore settings.
These tintinnids and calpionellids are used or have potential for biostratigraphic correlation with their parent rocks throughout the Tethyan Sea (Tappan 1993), being widespread from Australia (Brunnschweiler 1951) through Russia and Asia (Bugrova 2003), Iran and Morocco (Benzaggagh et al. 2010) in the east and across the Atlantic Ocean to Mexico in the west (Colom 1955, 1965), and in South Dakota, Wyoming, and Colorado (Eicher 1965) in central USA. Some of these fossils resemble modern species in their morphology and construction materials (Fig. 8.6). However, central in these debates about what fossils are tintinnids are the calcareous calpionellids. Calpionellids range from the late Tithonian, in the upper Jurassic, to the early Valanginian, in the lower Cretaceous (Tappan 1993; Tappan & Loeblich 1968; Remane 1985). Because of their cup-shaped loricae, calpionellids have commonly been assumed to be tintinnids. With this view, some taxonomists have interspersed calpionellids into modern families based on loric shape (Kofoid & Campbell 1939; Campbell 1954; Tappan & Loeblich 1968; Corliss 1979; Lynn 2008). Others have separated the calpionellids into their own tintinnid subgroup (see, e.g., Bonet 1956).

The central problem with identifying and describing calpionellids as tintinnids – either interspersing them into extant families or in their own taxon – is the composition of their loricae. Although all extant tintinnids have organic or agglutinated loricae (Chapter 3), calpionellid fossils are composed of calcite (Tappan 1993). Calpionellid fossils would then include the so-called calcareous tintinnids in the Paleozoic, Mesozoic, and Cenozoic. No modern classification separates the calpionellids into their own group.

Colom (1934, 1948) suggested that calcite may have replaced the original organic material during the fossilization process, but this seems highly unlikely as it would have had to occur in many fossils from many different rocks from many parts of the world (Tappan 1993). This difference in loric composition between extant ciliates and fossil calpionellids led Remane (1971, 1985) to declare that calpionellids are not tintinnids and not even ciliates.

With the current evidence, Remane’s (1971, 1985) view of a non-ciliate origin of calpionellids seems to be the most likely. That does not mean that sometime in the Jurassic a lineage of tintinnids evolved from making organic loricae to calcareous ones. This monophyletic group would then have gone extinct in the Cretaceous. However, no evidence exists for such an evolutionary scenario linking calpionellids with tintinnids.

Whatever calpionellids are – tintinnids or not – they do make excellent markers for use in biostratigraphy of upper Jurassic to lower Cretaceous rocks (Grün & Blau 1997; Remane 1985). Over the years, several schemes of zonal and subzonal divisions using calpionellids have been proposed (e.g., Altiner & Özkan 1991; Cantu-Chapa 1996; Grün & Blau 1996; Le Hégarat & Remane 1968; Pop 1976; Remane 1963, 1964, 1971; Remane et al. 1986). Grün & Blau’s (1997) latest calpionellid biochronology distinguishes six zones and 19 subzones (these zones also correlate with zonation developed for Mediterranean ammonites).

Genuine tintinnids, defined by having agglutinated loricae, occur in the Jurassic and Cretaceous (Tappan 1993). They have only one occurrence in the Jurassic (Rüst 1885) but are abundant in the shallow, neritic waters of the Cretaceous mid-continental seaway that flooded a central corridor through the center of North America from the Gulf of Mexico to the Arctic Ocean. In this environment, agglutinated tintinnids (Fig. 8.6) were abundant (Eicher 1965).

8.5 CENOZOIC TINTINNIDS: SPARSE

Tintinnids have been described from Paleocene, Eocene, and Oligocene marine deposits, and from Pleistocene lake sediments of the Cenozoic over the past 50 or so years by several authors (summarized by Tappan 1993; Bignot & Poignant 2010). Over 60 species (Figs 8.7–8.9) occur in the Paleogene rocks from the Gulf Coast of the USA, across Europe from Spain to Poland and Romania, and in Ukraine, Uzbekistan, and Turkmenistan (Tappan & Loeblich 1968; Tappan 1993; Bugrova 1983, 2003; Bignot & Poignant 2010). These occurrences are all in the ancient Tethys Seaway, an equatorial ocean that extended from the modern Gulf of Mexico region, across southern Europe and Asia, to Australia and southeast Asia. This seaway gradually closed as plate tectonic motions moved Africa and India into Europe and Asia, causing mountains to form, and oceanography to change in the rest of the world. The Tethys Seaway was a unique warm-water environment containing an abundance of fossil planktic and benthic organisms.

The tintinnids described from the ancient Tethyan marine rocks are mostly calcareous cup-shaped cones.
regions (Tappan & Loeblich 1968) that are not considered pseudarcellinids. Because no modern species of tintinnids are known to secrete CaCO₃, these also are unlikely to be tintinnids. The cups are mostly quite shallow and not deep; most are wider than they are long. Many of these might be larval-shelled invertebrates or other microbial eukaryotes.

8.6 ORGANIC AND AGGLUTINATED PHANEROZOIC FOSSILS: THE MOST LIKELY CANDIDATES

Tintinnids with organic loricae or loricae agglutinated with foreign particles gathered from the water column (Chapter 3), such as with coccoliths, silt, and clay attached to it, are abundantly preserved (Fig. 8.11) in some neritic marine sediments (Harman 1972; Echols & Fowler 1973), but they are uncommon in the fossil record. This dichotomy indicates that loricae cannot readily survive the processes associated with lithification of sediments into rocks. The oldest known tintinnid with an agglutinated organic lorica occurs in the Jurassic (Rust 1885). Others, placed in modern genera although this is likely incorrect, are abundant in the Cretaceous (Eicher 1965).
A gap in the occurrence of agglutinated loricae exists after the Cretaceous until they reappear in sediment cores that penetrate the Upper Pleistocene (Echols & Fowler 1973). Agglutinated and organic loricae are common in modern biotas in both open-ocean and neritic environments.

8.7 CONCLUSIONS AND PERSPECTIVES

The calcareous calpionellids and other forms may not be tintinnids at all. This view was common in the past when calpionellids were compared with a variety of microbial eukaryotes including thecameobians, foraminifera, and others. Some, especially species of Pseudarcella could even be small or larval invertebrate shells. Thus the reliable fossil record of tintinnids that can be used in calibrating molecular phylogenetic analyses is sparse and very incomplete. The first organic tintinnid from the Jurassic appears to be a fully developed form, thus indicating a previous undiscovered history.
Molecular data suggest that tintinnid classifications suffer from morphologic convergence of forms with dissimilar sequences, and some forms that are morphologically quite similar have distinct molecular sequences (Chapter 3). The fossil record of tintinnids does not provide a way to determine which of these convergences and divergences may be deciphered. Indeed, because of these molecular sequence data, no classification of the fossil tintinnids is attempted in this summary. Any such classification would be quite artificial.
8.8 KEY POINTS

1. Fossils normally leave few clues to their ciliate origin, except their shape and composition.
2. All putative tintinnids from the Precambrian and Proterozoic differ in shape, composition, or both from all known extant tintinnids, and are not tintinnids.
3. Calpionellids are likely not tintinnids because their composition differs from all known extant tintinnids.
4. The history of reliable tintinnids in the rock record is very incomplete, starting even with the first occurrence in the Jurassic, which indicates an even longer history.
5. Tintinnid classifications of both modern and fossil species are artificial and do not show evolutionary relationships.
6. Not only is more work necessary on the molecular phylogenetics of tintinnids, the fossil record must also be clarified and better documented with positive determinations that fill in the history of the group.

ACKNOWLEDGMENTS

We thank the editors of this book, especially John Dolan and Sabine Agatha. We also thank Alberto E. Patiño Douce, University of Georgia, for comments on the Mesoproterozoic Huangmailing Formation fossils. Funding came from the Alexander von Humboldt Foundation to M.D., and the Deutsche Forschungsgemeinschaft (grant STO414/3-1) to T.S. This is University of California Museum of Paleontology publication number 2033.